Sulfonamides (SAs) Rapid Test Kit

Technical Manual

(GICA)

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1 Principle and Application

The test kit is used for detecting Sulfonamides (SAs) in various samples, including tissues (from fish, shrimp, crab, livestock, and poultry), honey, milk, poultry eggs, water samples, and more.

The kit is developed using the principle of competitive colloidal gold Immunochromatographic Assay (GICA). After the sample solution is added to sample hole, if SAs is present, it will bind with gold labeled antibodies, thereby preventing the labeled antibodies from binding to the SAs conjugates on the nitrocellulose membrane.

If the content of SAs in sample solution is less than detection limit, it will make the test ("T") line colored, and the result is negative. If the content is greater than detection limit, no color reaction will be produced, and the result is positive.

2 Technique Data

Kit Sensitivity: Using Sulfamethazine (SM2) as the standard reference, the kit sensitivity is 5 ppb (ng/mL).

Detection Limits: (Using SM2 as the standard reference)

The detection limits for sulfonamides with this test card are as follows:

Drug name	Detection Limits(ppb)
Sulfamethazine(SM2)	5
Sulfamonomethoxine(SMM)	0.8
Sulfamethoxydiazine(SMD)	1
Sulfadoxine(SDM')	1.5
Sulfamerazine(SM1)	2
Sulfaguanidine (SG)	2
Sulfadiazine(SD/SDZ)	4
Sulfisomidine(SM2')	2.5
Sulfadimethoxine(SDM)	3
Sulfamethizole(SMT)	3
Sulfachloropyrazine(Esb3)	7.5
Sulfathiazole(ST)	9
Sulfachlorpyridazine(SCPA)	9
Sulfamethoxypyridazine(SMP)	9
Sulfaquinoxaline(SQX)	35
Sulfisoxazole(SIZ)	120
Sulfamethoxazole(SMZ)	120

Sample detection limit: (Using SM2 as the standard reference)

Tissue 5ppb

Honey 20ppb

Milk 40pbb

Urine 50ppb

Poultry egg 5ppb

Water 20ppb

3 Kit Content

Package specification	20T/Kit	40T/Kit
Test device (with dropper)	20	40
Sample reconstitution buffer	30mL×1	30mL×1
Instruction	1	1

4 Materials Required but Not Supplied

Equipment: grinder (for crushing solid samples), vortex mixer (for shake and mix), nitrogen evaporator, centrifuge, graduated transfer pipette, and balance with a division value of 0.01 g.

Micropipette: single-channel 20 to 200μL and 100 to 1000μL.

Reagents: Ethyl acetate, n-hexane.

5 Sample Pre-treatment

Please note that the labware must be clean. Use disposable droppers to avoid contamination of interference results.

5.1 Solution preparation before sample pre-treatment

Liquid 1: Acetonitrile-water solution

V(acetonitrile)/(water) = 84:16:

5.2 Sample pretreatment step:

5.2.1 Tissue:

(1) Weigh 4±0.05g of defatted, homogenized tissue sample into a 50mL centrifuge tube, add 2mL of purified water, and shake the sample into a paste. Then add 4mL of ethyl acetate, shake for 5 minutes, and centrifuge at room temperature at 4000 r/min or higher for 5 minutes.

(2) Transfer 2mL of the clear upper organic layer to a

clean glass tube and evaporate to dryness under nitrogen or air at 50-60°C.

(3) Add 0.5mL of sample reconstitution buffer to dissolve the dried residue, and set aside the lower layer of the liquid for testing.

Note: If there is a significant amount of residual oil after evaporation, first add 1-2mL of n-hexane, shake to mix well, then add 0.5mL of sample reconstitution buffer, mix well, and let it stand for about 5 minutes until the liquid layers are clearly separated. Collect the lower layer of the liquid for testing.

5.2.2 Honey:

(1) Weigh 1±0.05g of honey sample into a 15mL centrifuge tube, add 1mL of 0.5M hydrochloric acid, and incubate at 37°C for 30 minutes.

(2) Add 2.5mL of 0.2M sodium hydroxide (adjust pH to about 5), then add 4mL of ethyl acetate, shake for 5 minutes, and centrifuge at room temperature at 4000 r/min or higher for 10 minutes.

(3) Transfer 2mL of the clear upper organic layer to a clean glass tube and evaporate to dryness under nitrogen or air at 50-60°C.

(4) Add 0.5mL of sample reconstitution buffer to dissolve the dried residue, and set aside for testing.

5.2.3 Milk:

(1) Dilute fresh milk sample with deionized water at a 1:1 ratio, mix well, and set aside for testing.

5.2.4 Urine:

(1) Dilute fresh urine sample with deionized water at a 1:1 ratio, mix well, and set aside for testing.

5.2.5 Egg:

(1) Weigh 3±0.05g of homogenized egg sample into a 50mL centrifuge tube, add 9mL of acetonitrile-water solution, shake for 2 minutes, and centrifuge at 15°C at 4000 r/min for 10 minutes.

(2) Transfer 3mL of the upper layer to another centrifuge tube, add 3mL of deionized water, then add 4.5mL of ethyl acetate, shake for 1 minute, and centrifuge at 15° C at 4000 r/min for 10 minutes.

(3) Transfer all of the upper layer (until no more can be taken) to a 15mL centrifuge tube, and evaporate to dryness under nitrogen (or with a hairdryer) at 50-60°C.

(4) First add 1-2mL of n-hexane, shake to mix well, then add 0.3mL of sample reconstitution buffer, shake to dissolve the residue thoroughly, let it stand for about 5 minutes, and collect the lower layer of the liquid for testing.

5.2.6 Water:

(1) Collect clear water samples; if the sample is turbid, centrifuge at 4000 r/min for 10 minutes and collect the clear upper layer.

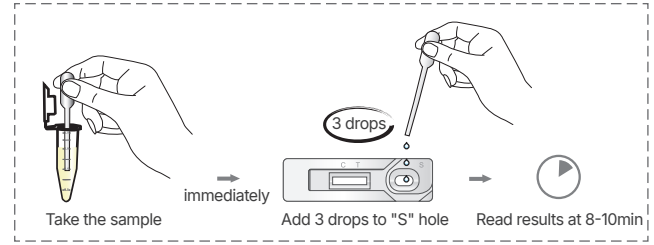
(2) Based on the detection limit of the sulfonamide drugs being tested, calculate the required dilution factor of the sample. Mix the appropriate amount of water sample with purified water and set aside for testing.

6 Test Steps

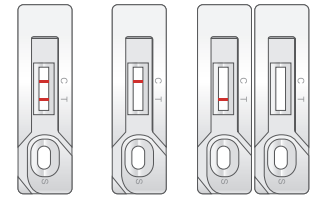
(1)Tear the foil pouch, take out of the test card, and put on a flat, clean work surface.

(2)Pipette the prepared sample solution with the provided dropper, then add 3 drops (approximately 60μL) vertically and slowly (Avoid the generation of bubbles) into the sample hole(“S”).

(3)Read the result at room temperature in 8-10 minutes. Results over 10 minutes can only be used as reference.



7 Results Judgement



Negative: Test(“T”) line and control(“C”) line both appear in the result window. It indicates that the concentration of SAs in the sample is below the detection limit, or absent.

Positive: Only control(“C”) line appears in the result window. It indicates that the concentration of SAs in the sample is above the detection limit.

Invalid: If the control(“C”) line does not appear, the result might be considered invalid.

8 Notice

8.1 Don’t use the expired or damaged products.

8.2 When the test card is taken out of the refrigerator, it should be restored to the room temperature and then opened. The opened test card should be used as soon as possible to avoid failure after being affected by moisture.

8.3 Avoid touching the white nitrocellulose membrane in the middle of the detection card.

8.4 In order to avoid cross-contamination, the droppers cannot pipet another Solution after pipetting one.

8.5 The sample solution to be examined needs to be clear and free of turbid particles. Otherwise, it is prone to lead to blockage, non-obvious color development and other abnormalities, affecting the determination of the experimental results.

9 Storage Conditions

The kit shall be stored at 2°C to 30°C (35.6°F to 86°F) in dry environment.

Shelf life: 12 months. The date of manufacture is presented in the label of the box.